Evidence for the Influence of Weeds on Corky Ringspot Persistence in Alfalfa and Scotch Spearmint Rotations

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ABSTRACT

Corky ringspot disease (CRS) of potato is caused by tobacco rattle virus (TRV). The virus is transmitted by the stubby root nematode (*Paratrichodorus allius*) in the Pacific Northwest potato-producing regions. Alfalfa (*Medicago sativa* L.) and Scotch spearmint (*Mentha cardiaca* Baker) rarely serve as hosts for TRV. Therefore, *P. allius* reared on these plants for 1 to 3 months are cleansed of TRV in greenhouse trials. However, weeds in alfalfa and Scotch spearmint rotation crops may serve as hosts for the virus. In greenhouse trials, hairy nightshade (*Solanum sarrachoides*), prickly lettuce (*Lactuca serriola*), henbit (*Lamium amplexicaule*) and, green foxtail (*Setaria viridis*) grown alone were found to be suitable hosts of *P. allius*, whereas Powell amaranth (*Amaranthus powellii*) was not. Viruliferous *P. allius* added to hairy nightshade, prickly lettuce, henbit, green foxtail, or Powell amaranth in mixtures with alfalfa and/or Scotch spearmint occasionally remained viruliferous over a 3- to 4-month period, whereas *P. allius* maintained on weed-free alfalfa or Scotch spearmint became virus-free after 1 to 2 months. Potato grown in soil containing *P. allius* that had been maintained on weed-alfalfa or weed-Scotch spearmint mixtures for 3 to 4 months exhibited slight to severe CRS symptoms on new tubers, whereas potato following weed-free Scotch spearmint or alfalfa were free from CRS symptoms. Severe CRS symptoms on potato tubers were only observed when potatoes were grown in soil containing *P. allius* that were maintained on hairy nightshade or hairy nightshade mixtures with alfalfa or Scotch spearmint. These preliminary data suggest that the presence of weeds that serve as hosts of both TRV and *P. allius* may nullify the positive effects of growing alfalfa or Scotch spearmint for CRS control. Targeted control efforts of known weed hosts may be required to successfully eliminate CRS from fields using alfalfa and Scotch spearmint rotational crops.

RESUMEN

El anillo corchoso de la papa (CRS) es causado por el tobaco rattle virus (TRV). El virus es transmitido por el nematodo de la raíz (*Paratrichodorus allius*) en regiones productores de papa del noroeste del Pacífico. La alfalfa (*Medicago sativa* L.) y menta escocesa (*Mentha cardiaca* Baker) rara vez sirven de hospedante para el TRV. Por lo tanto, en pruebas de crianza de *P. allius* en invernadero, en estas plantas por 1 a 3 meses sirvió para limpiarlo de TRV. Sin embargo, las malezas de los cultivos de rotación menta escocesa y alfalfa pueden servir como hospedantes para el virus. En pruebas de invernadero se encontró que la hierba mora (*Solanum sarrachoides*), la lechuga escarola (*Lactuca serriola*), la ortiga (*Lamium amplexicaule*) y el almorejo (*Setaria viridis*) crecidos separadamente son huéspedes apropiados de *P. allius*, mientras que el amaranto Powell (*Amaranthus powellii*) no se infectó. El *P. allius* virulífero colocado en hierba mora, escarola, ortiga, almorejo o

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ABBREVIATIONS: CRS, corky ringspot; ELISA, antigen coated plate enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; PNW, Pacific Northwest; TRV, tobacco rattle virus.

amaranto Powell en mezclas con alfalfa y/o menta escocesa, permanecieron ocasionalmente virulíferos por un periodo de 3 a 4 meses mientras que el *P. allius* mantenido en alfalfa o en menta escocesa libres de malezas, llegó a liberarse del virus después de 1 a 2 meses. Tubérculos nuevos de papa cultivada en suelos conteniendo *P. allius* que había sido mantenido sobre mezclas de malezas-alfalfa o de malezas-menta escocesa por 3 a 4 meses, mostraron ligeros a severos síntomas de CRS, mientras que la papa sembrada después de menta escocesa o de alfalfa libres de malezas no mostraron síntomas de CRS. Se observaron síntomas severos de CRS en tubérculos solamente cuando se sembró papa en terrenos que contenían *P. allius* mantenido en hierba mora o en mezclas de hierba mora con alfalfa o menta escocesa. Estos datos preliminares sugieren que la presencia de malezas que sirven como hospedantes tanto del TRV como del *P. allius* pueden anular los efectos positivos de sembrar alfalfa o menta escocesa para controlar CRS. Para eliminar exitosamente el CRS de los campos puede ser necesario hacer determinados esfuerzos orientados a eliminar las malezas que son huéspedes conocidos utilizando alfalfa y menta escocesa como cultivos de rotación.

INTRODUCTION

Corky ringspot (CRS) is a serious disease of potato (*Solanum tuberosum* L.) caused by tobacco rattle virus (TRV) and vectored by *Paratrichodorus allius* (Jensen) in the western U.S. (Mojtahedi et al. 2000). CRS causes necrotic arcs, rings, or spots in potato tubers, which can result in crop rejection. Surveys of Washington and Oregon potato fields indicate that the nematode vector is more prevalent than the virus (Mojtahedi and Santo 1999; Mojtahedi et al. 2000). The incidence of CRS in the Pacific Northwest (PNW) potato-growing regions has increased since first reported in Washington State in 1976 (Thomas et al. 1993). Soil fumigation with 1,3-dichloropropene prior to planting potatoes or oxamyl applied postemergence are the only tools currently available to growers to control or suppress *P. allius* populations (Ingham et al. 2000; Santo et al. 1997).

Like other plant-parasitic nematodes, *P. allius* molts several times during its life cycle and sheds the virus with each molt (Robinson and Harrison 1989). If young viruliferous nematodes are allowed to develop on a plant that is a non-host of TRV, the nematode population will become nonviruliferous after several generations. Rowe (1993) suggested that incidence of CRS in potato is reduced if the crop follows alfalfa implying that alfalfa was not a host of TRV. We have demonstrated in greenhouse trials that viruliferous *P. allius* populations were no longer able to transmit TRV to the susceptible indicator plant tobacco (*Nicotiana tabacum* L.) var. Samsun NN, after growing for several generations on alfalfa var. Vernema, or Scotch spearmint var. 770 (Mojtahedi et al. 2002b), suggesting that CRS could be cleansed from fields by growing these rotation crops. The impact of host crops of TRV on the epidemiology of CRS in the PNW was recently evaluated (Thomas et al. 1999; Mojtahedi et al. 2002a). Although crops such as alfalfa may help reduce CRS in soils, the presence of weeds that are hosts of TRV and *P. allius* may contribute to disease persistence.

The suitability of several crops and weeds as hosts for *P. allius* have been studied by various researchers (Allen and Davis 1982; Ayala et al. 1970; Brunt et al. 1996; Cooper and Harrison 1973; Davis and Allen 1975; Jensen et al. 1974; Lister and Murant 1967; Locatelli et al. 1978; Mojtahedi and Santo 1999; Mojtahedi et al. 2002a). Data compiled by Brunt et al. (1996) indicated that TRV has a wide host range including numerous weeds. Similarly, the influence of weeds on the CRS disease cycle has been investigated. Cooper and Harrison (1973) reported that spraing disease of potato (CRS) in plots kept free from weeds for 1.5 years was 3.4 times greater than in weed-infested plots. In contrast, French and Wilson (1976) reported that the incidence of CRS on potato was not affected by varying weed control in preceding barley (*Hordeum vulgare* L.) crops. However, TRV levels in weed seed was not reported in either study.

The primary interests of research on the impact of weeds on CRS are two fold. First, the weeds may serve as hosts for both TRV and *P. allius* to maintain the viruliferous vector population in the problem fields. Second, the virus may become seed-borne in susceptible weeds, which could disseminate and introduce TRV to potato fields that already contain the more widespread *P. allius* (Cooper and Harrison 1973; Lister and Murant 1967; Locatelli et al. 1978). Studies conducted in the PNW (Allen and Davis 1982; Davis and Allen 1975; Locatelli et al. 1978) demonstrated that several weed species were natu-

rally infected with TRV in commercial fields with CRS history. TRV was transferred from naturally infected weeds to tobacco by artificial inoculation, demonstrating that these weeds could possibly act as virus disease reservoirs in problem fields. In order to confirm the role of specific weeds in maintaining CRS in fields, it must be demonstrated that TRV can be acquired by a known nematode vector and transferred to susceptible weed hosts, and subsequently to potato. Consequently, the objective of these preliminary studies was to evaluate the role of selected weeds in maintaining TRV when present in mixed cultures with alfalfa or spearmint and to determine their role in the CRS disease cycle of subsequent potato crops.

MATERIALS AND METHODS

Five previously reported (Allen and Davis 1982; Jensen et al. 1974; Lister and Murant 1967; Locatelli et al. 1978) weed hosts of TRV, namely henbit (*Lamium amplexicaule* L.), green foxtail (*Setaria viridis* (L.) Beauv.), prickly lettuce (*Lactuca serriola* L.), Powell amaranth (*Amaranthus powellii* S. Wats.), and hairy nightshade (*Solanum sarrachoides* Sendtner) were each grown separately and with alfalfa (var. Vernema) and/or Scotch spearmint (var. 770) in greenhouse pots over a 3- or 4-month period. A loamy sand soil (83.1% sand, 15% silt, and 1.9% clay) was prepared and fumigated with methyl bromide (0.3 kg/m^3) several weeks prior to each experiment.

In selected treatments, ten alfalfa seeds or four, 4-cm-long spearmint rhizomes were planted in potting soil in 6.6-L plastic pots and grown in a greenhouse held at 25 ± 4 C. Day length was extended to 16 h with sodium vapor lamps delivering 650 uE/m^{2}sec. Each pot was thinned to four seedlings 1 wk after planting. Weed seedlings or seed were planted alone or interseeded with alfalfa or spearmint in selected treatments. Prickly lettuce and henbit seedlings at the two- to three-leaf stage were transplanted from plants growing locally in fields with no history of CRS, whereas seed was used to establish green foxtail, Powell amaranth, and hairy nightshade. Roots of prickly lettuce and henbit seedlings were rigorously washed with water prior to transplanting to remove any nematodes that might be present. Roots were tested for TRV using ELISA (described below) to ensure they were virus-free prior to transplanting. All weeds were thinned to four uniform plants per pot at 1 wk after seeding or transplanting. A control treatment of tobacco, var. Samsun NN, a host of *P. allius* and TRV and an indicator plant, was also included (Mojtahedi and Santo 1999). Pots were fertilized with 10 g of Osmocote slow release fertilizer (N=14%, P=14%, K=14% from Scott-Sierra Horticultural Products Co., Marysville, OH).

Two weeks after seeding or transplanting, pots were inoculated with 150 viruliferous *P. allius* in 5 mL of water pipetted into three holes in each pot. Viruliferous *P. allius* were obtained from isolates collected from a CRS problem field near Pasco, WA, and maintained on tobacco (Mojtahedi et al. 2001). All pots were maintained at field capacity (9% moisture by weight) to increase the vector population (Mojtahedi and Santo 1999).

At 1, 2, 3, and 4 months after addition of viruliferous nematodes, four 2.5-cm-diameter soil cores containing a total of 250 cm^3 soil were removed from each pot and nematodes were isolated by sieving and sugar-centrifugal flotation (Jenkins 1964). Nematodes were counted and the reproductive factor (RF) was determined using the formula $RF = P_f/P_i$, where P_f = final nematode count and P_i = initial inoculum (O'ostenbrink 1966). Nematodes were then bioassayed for TRV by pipetting into soil in 13-cm-diameter clay pots containing one tobacco seedling. After 3 wk, visual TRV symptoms on tobacco were recorded and TRV presence on tobacco roots were confirmed by ELISA (Converse and Martin 1990). The antibody used was PVAS 820 from the American Type Culture Collection (ATCC) Rockville, MD. Root and shoot tissue of each species growing alone in 6.6-L pots were also tested for presence of TRV at each sampling date by ELISA and (or) RT-PCR (Crosslin and Thomas 1995). ELISA tests for TRV were considered positive if optical density reading was ≥ 0.1 and at least twice that of uninoculated control plants of the same species (Sutula et al 1986). Optical density ranges for healthy controls of each plant species were tobacco (-0.005 to 0.03), alfalfa (-0.29 to -0.28), spearmint (0.04 to 0.26), henbit (-0.004 to 0.05), prickly lettuce (-0.30 to -0.13), *P. amaranth* (-0.007 to 0.05), and green foxtail (-0.35 to 0.015).

As a final test, each 6.6-L pot was bioassayed with potato following the last sampling date in each experiment to determine if TRV could be transmitted from the host plants by *P. allius* and cause CRS symptoms on potato. Plant roots and shoots were removed from the soil in each 6.6-L pot and certified seed of potato, var. Russet Burbank, tubers were planted. All newly formed potato tubers were harvested after 10- to14-wk growth in the greenhouse. Tubers were sectioned longitu-

dinally into four wedges and each cut surface examined for CRS symptoms using a visual scale of 0 = no symptoms to 8 = all eight surfaces were blemished.

Each treatment was replicated five times in a completely randomized design. Experiment 1 was conducted with alfalfa as the "cleansing crop" and included henbit, prickly lettuce, Powell amaranth, and green foxtail as weed hosts. Treatments consisted of alfalfa alone, alfalfa plus each individual weed, each weed alone, and tobacco (Table 1). Experiment 2 was conducted with Scotch spearmint as the cleansing crop and included the same four weeds and crop-weed combinations (Table 2).

Experiment 3 was conducted using both alfalfa and Scotch spearmint as cleansing crops, and hairy nightshade as a weed host of *P. allius* and TRV (Table 3). Hairy nightshade was previously found to be a host of both *P. allius* and TRV (Allen and Davis 1982; Jensen et al. 1974; Mojtahedi et al. 2004). Treatments consisted of alfalfa alone, Scotch spearmint alone, alfalfa plus hairy nightshade, Scotch spearmint plus hairy nightshade, hairy nightshade alone, and tobacco. In experiment 3, only three monthly sampling dates were employed.

RESULTS AND DISCUSSION

In all experiments, tobacco was shown to be an excellent host of *P. allius* (RF > 1) and remained a reservoir for TRV throughout the 4-month sampling period (Figure 1a and Tables 1, 2, and 3). Viruliferous *P. allius* extracted from tobacco transmitted the virus to healthy tobacco or potato plants in all experiments (Tables 1, 2, and 3). As a result, tobacco was used as a positive control.

Experiment 1—Alfalfa Interplanted with Four Weed Species

Alfalfa maintained *P. allius* populations (RF > 1), at all sampling dates except the first (RF = 0.77) (Figure 1a), and nematodes extracted from alfalfa at all sampling dates were unable to transmit TRV to indicator tobacco seedlings (Table 1). Similar results were reported by Mojtahedi et al. (2002b). Powell amaranth was not a suitable host for *P. allius* because nematode populations declined in all studies (Figure 1b). Henbit seedlings often died several months after transplanting and *P. allius* populations declined on henbit at the final sampling date after initially increasing (RF = 8.8 ± 4.3 to 0.06 ± 0.06) (Figure 1b). Populations of *P. allius* were maintained on prickly

TABLE 1—*Detection of tobacco rattle virus (TRV) by ELISA in roots and/or shoots of alfalfa and four weed species over a 4-month sampling period after inoculating with 150 viruliferous* Paratrichodorus allius *and detection of TRV by ELISA in tobacco that received nematodes extracted from pots containing alfalfa, four weed species, or alfalfa-weed mixtures over a 4-month sampling period.*

	1 month		2 month		3 month		4 month	
	No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')	
Host plant(s)	Host plant	Indicator tobacco	Host plant	Indicator tobacco	Host plant	Indicator tobacco	Host plant	Indicator tobacco
Tobacco	5/5 (2.4-0.1)	2/5 (1.3- 0.1)	5/5 (1.5-0.1)	5/5 (0.5- 0.1)	5/5 (0.3-0.1)	3/5 (0.3-0.2)	4/5 (0.1-0.1)	5/5 (0.4-0.1)
Alfalfa	0/5	0/4	0/5	0/5	0/5	0/5	0/5	0/5
Henbit	2/5 (0.3-0.1)	0/5	3/5 (0.5-0.1)	2/4 (0.6-0.6)	3/5 (2.4-0.1)	1/5 (0.1)	0/2	0/1
Prickly lettuce	0/5	0/5	0/5	0/5	0/5	1/5 (0.6)	0/5	2/5 (1.3-0.1)
P. amaranth	0/4	0/2	1/5 (0.1)	**	0/5	0/1	0/5	**
Green foxtail	0/5	0/2	0/5	0/4	0/5	0/4	0/5	0/4
Alfalfa + henbit	*	0/5	*	0/5	*	0/5	*	0/5
Alfalfa + prickly lettuce	*	1/5 (0.1)	*	0/5	*	0/5	*	0/5
Alfalfa + P. amaranth	*	0/1	*	0/4	*	0/5	*	0/5
Alfalfa + green foxtail	*	0/5	*	0/5	*	0/5	*	0/5

* no data obtained.

** no nematodes were detected in soil samples and tobacco bioassay was not conducted.

lettuce at all sampling dates and on green foxtail at the 2-, 3-, and 4-month sampling dates (Figure 1b). These two weed species are suitable hosts for *P. allius*. *Paratrichodorus allius* populations were maintained and increased in pots containing mixtures of alfalfa and each of the four weed species, except the initial sampling date from alfalfa plus Powell amaranth which resulted in an RF < 1 (Figure 1c).

Approximately half of the henbit plants grown alone tested positive for TRV throughout the first three sampling periods when *P. allius* populations remained high (Table 1). TRV detected by ELISA was usually confined to root tissue, but virus was also located in shoot tissue of henbit on the third sampling date. *Paratrichodorus allius* extracted from henbit at the second and third sampling dates were able to transmit TRV to tobacco (Table 1). However, no TRV was detected in tobacco that received *P. allius* extracted from pots containing a mix of henbit and alfalfa at any sampling date. Similarly, no CRS symptoms were present on potato tubers that were grown in soil containing *P. allius* from pots containing mixes of henbit and alfalfa (Table 4). The difficultly in maintaining live henbit plants in mixes with alfalfa throughout the duration of the experiment may have contributed to the lack of henbit acting as a virus reservoir in mixes with alfalfa. Henbit may be particularly important in the epidemiology of CRS as TRV was transmitted via seed to new seedlings when henbit plants were artificially inoculated with TRV (Lister and Murant 1967).

No TRV was detected by ELISA in prickly lettuce evaluated from all four sampling dates (Table 1). However, TRV was detected in 20% to 40% of tobacco that received *P. allius* extracted from pots containing prickly lettuce at the three- and four- month sampling dates (Table 1). These data suggest that prickly lettuce grown alone remained a reservoir for TRV even though levels were too low to detect with ELISA. TRV was detected in *Lactuca* sp., assumed to be prickly lettuce, by ELISA in studies by Allen and Davis (1982) and in prickly lettuce roots in studies by Mojtahedi et al. (2004). TRV was detected in only one tobacco plant at the first sampling date that was inoculated with *P. allius* extracted from pots containing mixes of prickly lettuce and alfalfa. However, slight CRS-like symptoms were present on potato tubers that were grown in soil containing *P. allius* from pots containing mixes of prickly lettuce and alfalfa. Low levels of TRV may have been maintained in pots containing prickly lettuce, allowing *P. allius* to acquire and transmit the virus to potato in a very low frequency.

TRV was detected only once by ELISA in Powell amaranth root tissue at the second sampling date (Table 1). No

TABLE 2—*Detection of tobacco rattle virus (TRV) by ELISA in roots and/or shoots of Scotch spearmint and four weed species over a 4-month sampling period after inoculating with 150 viruliferous* Paratrichodorus allius *and detection of TRV by ELISA in tobacco that received nematodes extracted from pots containing Scotch spearmint, four weeds, or Scotch spearmint-weed mixtures over a 4-month sampling period.*

	1 month		2 month		3 month		4 month	
	No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')	
Host plant(s)	Host plant	Indicator tobacco	Host plant	Indicator tobacco	Host plant	Indicator tobacco	Host plant	Indicator tobacco
Tobacco	5/5 *(1.7-0.3)*	3/5 *(2.0-1.1)*	4/5 *(0.2-0.1)*	4/5 *(1.0-0.3)*	5/5 *(0.3-0.1)*	3/5 *(1.3-0.1)*	5/5 *(0.2-0.1)*	5/5 *(0.7-0.2)*
Scotch spearmint	0/5	0/5	0/5	0/4	0/5	0/4	0/5	0/5
Henbit	0/4	0/4	0/5	0/2	1/5 (0.1)	0/1	0/5	**
Prickly lettuce	0/5	0/5	0/5	0/5	0/5	2/5 *(2.5-2.4)*	0/5	2/5
P. amaranth	0/5	**	0/5	**	0/5	**	0/5	**
Green foxtail	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5 (0.1)
Spearmint + henbit	*	0/4	*	0/4	*	0/3	*	0/1
Spearmint + prickly lettuce	*	1/5 *(0.8)*	*	0/5	*	2/5 *(1.9-1.7)*	*	0/5
Spearmint + P. amaranth	*	0/4	*	0/5	*	0/5	*	0/5
Spearmint + green foxtail	*	0/5	*	0/5	*	0/5	*	0/5

* no data obtained.

** no nematodes were detected in soil samples and tobacco bioassay was not conducted.

TRV was detected in tobacco that received *P. allius* extracted from pots containing Powell amaranth (alone or in mixes with alfalfa) at any sampling date. However, slight CRS-like symptoms were present on one of three potato tubers in one pot that were grown in soil containing *P. allius* from mixes of Powell amaranth and alfalfa. Low levels of TRV were possibly maintained in pots containing Powell amaranth with alfalfa, allowing *P. allius* to acquire and transmit TRV from Powell amaranth to potato and cause slight symptoms on tubers.

Although *P. allius* populations declined in pure cultures of Powell amaranth, nematodes thrived in mixtures of alfalfa and Powell amaranth, and *P. allius* may have acquired TRV by incidental probing of Powell amaranth roots. TRV was acquired by Powell amaranth from viruliferous *Trichodorus teres*, Hooper in previous studies (Jensen et al. 1974). Although a poor host for the *P. allius* in these studies, Powell amaranth may act as a TRV reservoir and incidental feeding by *P. allius* on the weed may transfer the virus to other hosts including potato.

No TRV was detected by ELISA in green foxtail at the four sampling dates (Table 1). Likewise, no TRV was detected in tobacco that received *P. allius* extracted from pots containing green foxtail (alone or in mixes with alfalfa) at any sampling date (Table 1). However, slight CRS-like symptoms were present on a few potato tubers in one pot that were grown in soil containing *P. allius* reared on mixes of green foxtail and alfalfa. Low levels of TRV may have been maintained in pots containing green foxtail with alfalfa. Whether the alfalfa or green foxtail grown prior to potatoes contributed to these

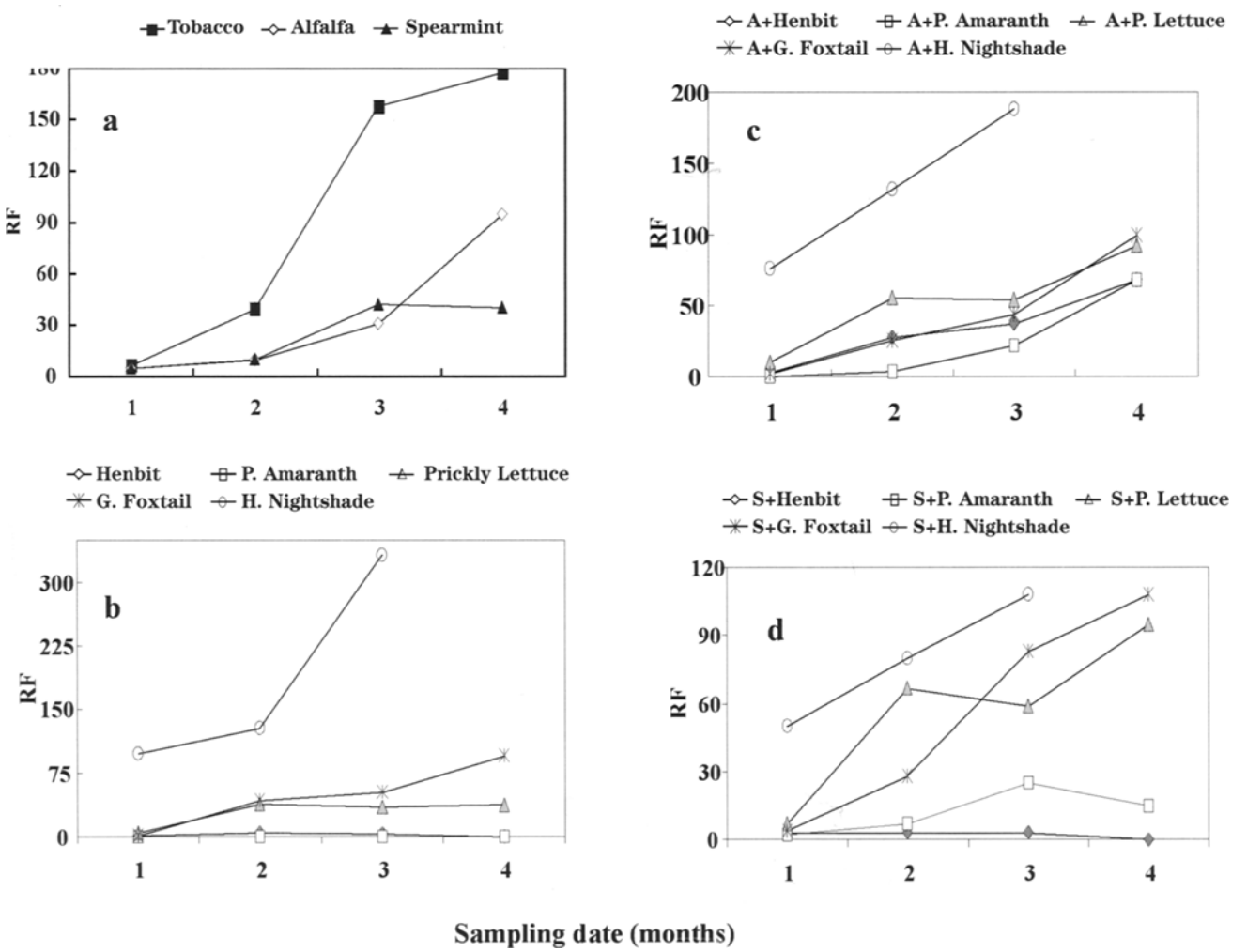

FIGURE 1.
Reproductive factor (RF = final nematode count/initial nematode inoculum) of *Paratrichodorus allius* on tobacco, alfalfa, and Scotch spearmint (1a); five weed species (1b); and crop-weed mixtures (A=alfalfa and S=spearmint) (1c and 1d).

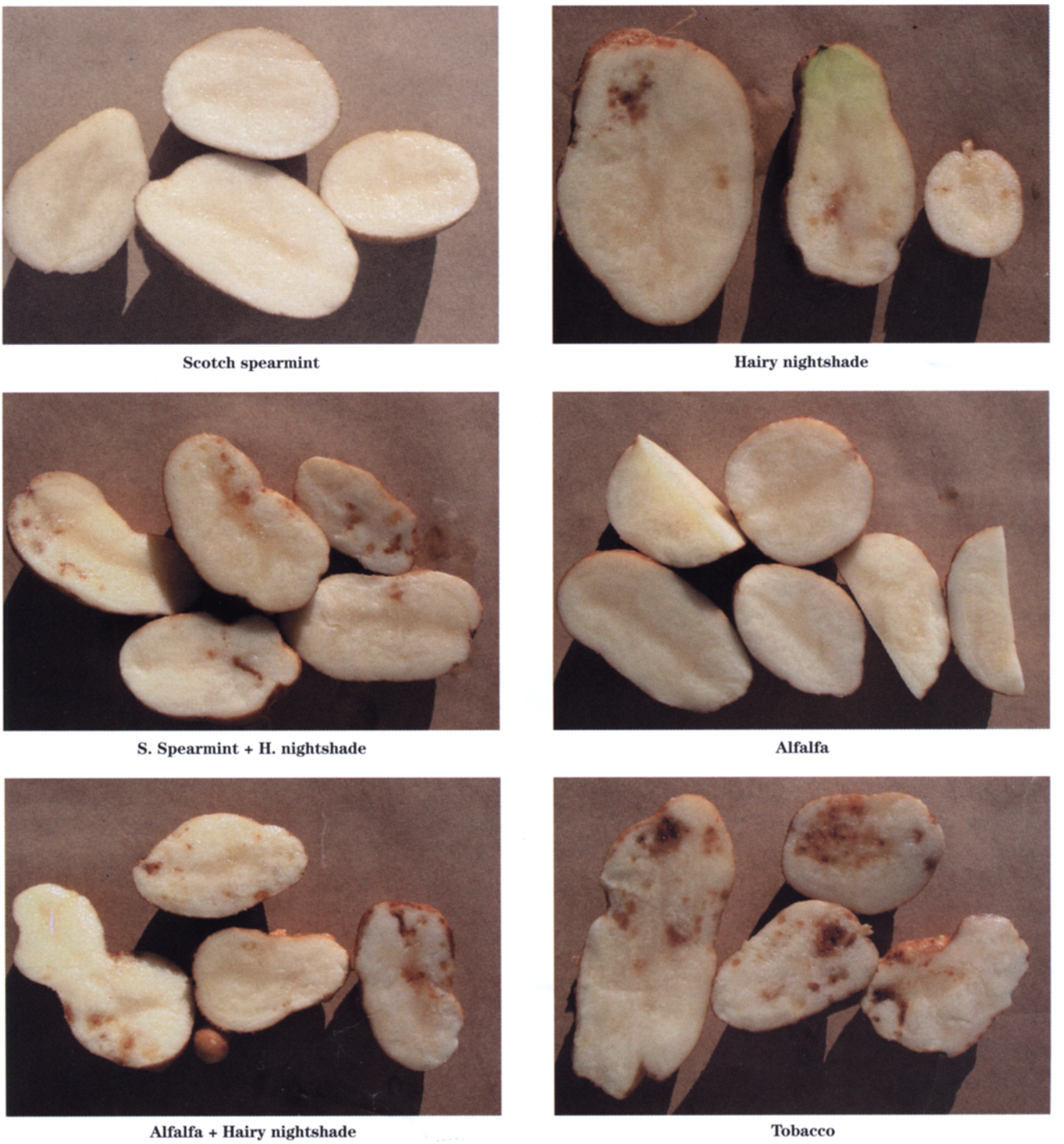

FIGURE 2.
CRS symptoms on potato tubers grown in soil containing *P. allius*, which was maintained for 3 months on host plants of Scotch spearmint, hairy nightshade, a mix of Scotch spearmint and hairy nightshade, alfalfa, a mix of alfalfa and hairy nightshade, and tobacco.

CRS-like symptoms cannot be determined from these studies. However, similar CRS-like symptoms were noted occasionally when potatoes followed alfalfa alone in experiment 3 (Table 4). No TRV was detected by ELISA in green foxtail subjected to viruliferous *P. allius* for 55 days in a previous study (Mojtahedi et al. 2004), but TRV was detected in green foxtail collected from fields with known CRS history (Allen and Davis 1982).

Experiment 2—Scotch Spearmint Interplanted with Four Weed Species

Scotch spearmint maintained *P. allius* populations that were cleansed of TRV within 1 month after inoculation to Scotch spearmint (Figure 1a and Table 2). *Paratrichodorus allius* extracted from Scotch spearmint at all sampling dates did not transmit TRV when added to indicator tobacco seedlings or potato (Tables 2 and 4). These results are in agreement with Mojtahedi et al. (2002b), suggesting Scotch spearmint as a rotation crop to suppress CRS. *Paratrichodorus allius* populations declined on Powell amaranth and henbit (RF < 1), but were maintained on green foxtail and prickly lettuce throughout the 4-month sampling period (Figure 1b). *Paratrichodorus allius* populations increased on all Scotch spearmint-weed mixtures except with henbit, in which populations declined after the 2-month sample date (Figure 1d).

Henbit grown alone remained a reservoir for TRV, as TRV was detected by ELISA in henbit root tissue of one of five plants at the 3-month sampling (Table 2). *Paratichodorus allius* extracted from henbit pots did not transmit TRV to tobacco at any sampling dates (Table 2). Similarly, no TRV was detected in tobacco that received *P. allius* extracted from pots containing mixtures of henbit and Scotch spearmint at any sampling date (Table 2). In addition, no CRS symptoms were present on potato tubers that were grown in soil harboring *P. allius* from pots containing mixes of henbit and Scotch spearmint (Table 4). As in experiment 1 with alfalfa, the difficultly in maintaining live henbit plants in mixes with Scotch spearmint and declining nematode populations throughout the duration of the experiment likely contributed to the lack of CRS symptoms in potato.

No TRV was detected by ELISA in prickly lettuce at any sampling date (Table 2). However, TRV was detected in 40% of tobacco that received *P. allius* extracted from pots containing prickly lettuce at the 3- and 4-month sampling dates (Table 2). Apparently, prickly lettuce remained a reservoir for TRV even though levels were too low to detect in prickly lettuce tissue with ELISA. TRV was also detected in tobacco that received *P. allius* extracted from pots containing mixes of prickly lettuce and Scotch spearmint at the 1- and 3-month sampling dates (Table 2). However, no CRS symptoms were present on potato tubers that were grown in soil harboring *P. allius* from pots containing mixes of prickly lettuce and Scotch spearmint (Table 4). The number of viruliferous *P. allius* must have been below the damage threshold (3/250 cm^3) reported for this nematode (Mojtahedi et al. 2001).

No TRV was detected by ELISA in Powell amaranth at all four sampling dates (Table 2). Nematode populations were not adequate in pots containing only Powell amaranth to extract and bioassay on tobacco. However, nematode populations were maintained in pots containing mixes of Powell amaranth and Scotch spearmint, and no TRV was detected in tobacco that received *P. allius* extracted from those pots (Figure 1d and Table 2). Similarly, no CRS symptoms were observed on potato tubers grown in soil containing *P. allius* from mixes of Powell amaranth and Scotch spearmint (Table 4).

Similar to results in experiment 1, no TRV was detected by ELISA in green foxtail at all four sampling dates (Table 2). However, TRV was detected in one tobacco that received *P. allius* extracted from pots containing green foxtail alone at the fourth sampling date (Table 2). Slight CRS-like symptoms were present on one potato tuber grown in soil containing *P. allius* reared on mixes of green foxtail and Scotch spearmint, indicating that low levels of TRV may have been maintained in pots containing green foxtail with Scotch spearmint (Table 4).

In experiments 1 and 2, of the four weed species tested, only nematodes extracted from pots with mixes of prickly lettuce and Scotch spearmint or alfalfa were able to transmit TRV to tobacco (Tables 1 and 2). Nematodes extracted from pots containing one of these four weeds in mixtures with alfalfa or Scotch spearmint were occasionally able to transmit TRV to potato resulting in very slight CRS symptoms on tubers. Thus, these four weeds likely play a minor role in the epidemiology of CRS, but may perpetuate TRV and its nematode vector in problem fields.

TABLE 3—*Detection of tobacco rattle virus (TRV) by ELISA in roots and/or shoots of alfalfa, Scotch spearmint, and hairy nightshade over a 3-month sampling period after inoculating with 150 viruliferous* Paratrichodorus allius *and detection of TRV by ELISA in tobacco that received nematodes extracted from pots containing alfalfa, Scotch spearmint, hairy nightshade, or mixtures of crops with hairy nightshade over a 3-month sampling period.*

	1 month		2 month		3 month	
	No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')		No. TRV (+)/no. tested (OD range of TRV '+')	
Host plant(s)	Host plant	Indicator tobacco	Host plant	Indicator tobacco	Host plant	Indicator tobacco
Tobacco	4/5 (1.1-0.1)	3/5 (1.2-0.1)	5/5 (2.5-0.4)	5/5 (1.1-0.1)	4/5 (2.3-0.1)	5/5 (1.7-0.5)
Alfalfa	0/5	1/5 (0.4)	0/5	0/5	0/5	0/5
S. Spearmint	0/5	5/5 (0.6-0.5)	0/5	0/5	0/5	0/4
H. Nightshade	0/5	3/5 (2.1-1.0)	5/5 (0.5-0.1)	5/5 (1.2-0.3)	3/5 (0.4-0.3)	5/5 (1.5-0.4)
Alfalfa + H. nightshade	*	3/5 (1.6-0.3)	*	4/5 (1.2-0.4)	*	5/5 (2.1-0.3)
Spearmint + H. nightshade	*	5/5 (1.4-0.1)	*	3/5 (1.0-0.1)	*	3/5 (2.5-1.0)

* no data obtained.

TABLE 4—*Corky ringspot disease incidence and severity on Russet Burbank potato tubers grown in soil with different crop and weed histories.*

Previous crop	Previous weed	Corky ringspot disease symptoms in potato tubers* Mean Percent Incidence	Severity***
Experiment 1			
Tobacco	None	97 ± 3.3 (18)**	6.6 ± 2.0
Alfalfa	None	0 ± 0 (21)	0 ± 0
Alfalfa	Henbit	0 ± 0 (11)	0 ± 0
Alfalfa	Prickly lettuce	3 ± 4.2 (15)	0.1 ± 0.2
Alfalfa	Powell amaranth	7 ± 6.7 (13)	0.2 ± 0.4
Alfalfa	Green foxtail	6 ± 7.1 (11)	0.1 ± 0.2
Experiment 2			
Tobacco	None	73 ± 19.5 (16)	5.4 ± 3.1
Scotch spearmint	None	0 ± 0 (20)	0 ± 0
Scotch spearmint	Henbit	0 ± 0 (3)	0 ± 0
Scotch spearmint	Prickly lettuce	0 ± 0 (8)	0 ± 0
Scotch spearmint	Powell amaranth	0 ± 0 (32)	0 ± 0
Scotch spearmint	Green foxtail	5 ± 5.0 (32)	0.2 ± 0.4
Experiment 3			
Tobacco	None	77 ± 9.8 (36)	4.8 ± 2.3
None	Hairy nightshade	72 ± 13.2 (32)	4.5 ± 2.6
Alfalfa	None	8 ± 5.3 (37)	0.2 ± 0.2
Alfalfa	Hairy nightshade	88 ± 5.2 (20)	6.7 ± 1.5
Scotch spearmint	None	0 ± 0 (42)	0 ± 0
Scotch spearmint	Hairy nightshade	65 ± 15.0 (29)	4.0 ± 1.9

*Numbers following treatment means represent standard error.

**Total number of tubers examined.

***Tubers were sectioned longitudinally into four wedges and each cut surface examined for CRS symptoms using a visual scale of 0 = no symptoms to 8 = all eight surfaces were blemished.

Experiment 3—Alfalfa and Scotch Spearmint Interplanted with Hairy Nightshade

Paratrichodorus allius populations increased with time on all host plant species (tobacco, alfalfa, Scotch spearmint, and hairy nightshade) in experiment 3 (Figure 1). Hairy nightshade was a particularly good host of *P. allius* (RF > 300 at 3 months) (Figure 1b). *Paratrichodorus allius* extracted from alfalfa pots did not transmit TRV to indicator tobacco seedlings at the 2- or 3-month sample dates (Table 3). However, very slight CRS-like symptoms were observed on two potato tubers grown in presence of nematodes that were maintained on alfalfa for 3 months (Table 4). Occasionally, low levels of TRV can be detected in alfalfa using RT-PCR after growing for several months in the presence of viruliferous *P. allius* (Crosslin unpublished). Subsequent testing of these two slightly symptomatic tubers with RT-PCR was negative for presence of TRV. We suspect the tuber symptoms observed were a physiological disorder rather than CRS.

ELISA optical density readings for Scotch spearmint shoots and roots were slightly >0.1, but less than twice the virus-free controls at several sampling dates, indicating a possible background noise. The absence of TRV in Scotch spearmint was confirmed with RT-PCR. *Paratrichodorus allius* extracted from Scotch spearmint pots 1 month after inoculation were able to transmit TRV when added to tobacco (Table 3). However, TRV was not transmitted by *P. allius* to tobacco when extracted from Scotch spearmint at the 2- or 3-month sample dates (Table 3). Likewise, no TRV symptoms appeared on potato tubers grown in soil containing *P. allius* that had been maintained on Scotch spearmint for 3 months, confirming that the nematode population was cleansed after being maintained on Scotch spearmint for over a month (Figure 2 and Table 4).

Paratrichodorus allius populations were maintained on hairy nightshade grown alone or in mixtures with Scotch spearmint or alfalfa (Figure 1b, 1c, and 1d). Sixty to 100 percent of hairy nightshade root tissue tested positive for TRV at the 2- and 3-month sampling dates (Table 3). Hairy nightshade root, shoot, and fruit tested positive for TRV in previous studies (Allen and Davis 1982), but TRV (as detected by ELISA) was confined to root tissue of hairy nightshade in this study.

Paratrichodorus allius isolated from pots containing hairy nightshade alone or in mixtures with Scotch spearmint or alfalfa were able to transmit TRV to tobacco at all sampling periods (Table 3). Potato planted in pots containing *P. allius* previously grown in the presence of hairy nightshade or mixes of hairy nightshade with alfalfa or Scotch spearmint exhibited severe CRS symptoms on tubers (Table 4 and Figure 2). Hairy nightshade was reported to be a good host of TRV and the nematode vector (Allen and Davis 1982; Jensen et al. 1974; Mojtahedi et al. 2004) and may be particularly important for growers to target in weed control programs in fields with a history of CRS.

Prickly lettuce, green foxtail, and Powell amaranth grown in combination with alfalfa and/or Scotch spearmint were occasionally able to maintain sufficient quantities of TRV that were transmitted to potato via *P. allius* resulting in slight CRS symptoms in tubers. However, their impact was far less than hairy nightshade according to the lower incidence and severity of CRS on potato that followed them.

In order to utilize crop rotation to suppress CRS, specific weeds that serve as reservoirs of TRV and transmitted to potato via *P. allius* should be identified and controlled. In these studies hairy nightshade was a particularly good host of both the nematode and TRV. Hairy nightshade is often difficult to control in potatoes and is a common weed in potato rotations in the western U.S. However, several effective herbicides are available for hairy nightshade control in alfalfa (bromoxynil, 2,4D-B, diuron, EPTC, hexazinone, imazethapyr, norflurazon, and terbacil) and Scotch spearmint (bentazon, bromoxynil, diuron, terbacil, pyridate). Multiple hay cuttings and the lack of soil disturbance by cultivation would also serve to suppress hairy nightshade in alfalfa and Scotch spearmint.

Utilizing crop rotation to suppress CRS disease is a desirable strategy that in theory, could reduce the need for costly soil fumigation. Crops like alfalfa and Scotch spearmint can be utilized in rotation with potato to suppress CRS disease. However, our results illustrate that specific weeds may act as TRV reservoirs and nullify the beneficial effect of these rotational crops. We have provided initial evidence that *P. allius* maintained on weed hosts of TRV can retain the virus and transmit it to potato resulting in CRS symptoms.

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